

# Myosin Light Chain-1 is Associated with Beef Carcass Tenderness

Undergraduate Honors Research Project

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## **Abstract**

Flavor and tenderness are the most desirable qualities that serve to distinguish beef products. Furthermore, inconsistent tenderness has a significantly negative economic impact on the beef industry. In a follow-up study to Sawdy, Kaiser, St-Pierre & Wick (2004), bovine myosin light chain 1 (bMLC1) concentration, in samples derived from ribeye steaks at 36 h postmortem, was determined to be positively associated with Warner-Bratzler shear values in matched ribeye steaks aged 7 days. Additionally, the band intensity of bMLC1 was correlated with two distinct myosin heavy chain (MyHC) fragments that previously were determined to be positively associated with tenderness. The results of this investigation establish the foundation for a method to identify gene products with positive and negative associations with tenderness. This methodology will be useful in the design of breeding programs for the improvement of beef palatability. In addition, the findings of this study give rise to a potential mechanism that is responsible for regulating beef carcass tenderness.

*Keywords:* Tenderness; Proteomic fingerprint; Myofibril; Image analysis; Bovine myosin light chain 1

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## **Introduction**

Processors and consumers report that flavor and tenderness are the most desirable qualities that serve to distinguish beef products (Koohmaraie, 1992; Roeber, Belk, Savell, Morgan, Montgomery, & Smith, 2001; Smith, et al., 1996). Further reports indicate that inconsistent tenderness has a significantly negative economic impact on the beef industry and that consumers are willing to pay a premium price for beef guaranteed to be flavorful and tender (Mintert, Lusk, Schroeder, Fox, & Koohmaraie, 2000; Boleman, et al., 1997). Along with environmental contributors and age of the animal, genetic differences among cattle dictate the degree and variability of tenderness.

Although delivering a tender and palatable product to consumers is the main objective of the beef industry, current efforts to separate carcasses based on tenderness are less than sufficient (Smith et al., 1996; Brooks et al., 2000; Roeber et al., 2001). These attempts rely solely on the USDA beef quality grading system, which uses marbling scores taken at 36 h postmortem as one of the chief traits to judge tenderness and predict quality, including tenderness, after “aging” from 7-21 d. While marbling affects palatability, its relationship to tenderness is controversial, and it is not known to be highly predictive of tenderness. This means that the current grading system is not allowing the beef industry to take full advantage of the chance to capitalize on the consumers’ willingness to pay higher prices for tender beef (Savell et al. 1987). Thus, the current inability to separate carcasses into tenderness categories permits “inadequate tenderness” to remain what beef producers and processors describe as the leading problem facing the beef industry today and in the last fifteen years (Morgan et al. 1991; Smith, Savell, Morgan, & Montgomery, 2000).

The ability to predict tenderness accurately is inherently confounded by the process of beef carcass fabrication. As mentioned earlier, beef carcasses in large processing facilities are usually quality-graded based on intramuscular fat content at 36-48 h post-harvest. Furthermore, almost immediately after grading, the carcasses are fabricated into wholesale and retail cuts, packaged, and prepared for distribution. Once the carcass has been fabricated, tracing the cuts from individual animals proves to be enormously uneconomical. Consequently, there is a critical need in the beef industry for an accurate, rapid, and reliable method to predict the tenderness of individual beef carcasses prior to fabrication. Until such a method is available, cattle producers and processors will continue to be unable to benefit from the economic benefits that could be generated from such information in marketing beef products to consumers.

Biochemical events within skeletal muscle during the post-harvest aging process contribute to the structural breakdown of muscle fiber integrity over time such that, to a certain degree, the longer a carcass is aged, the more tender the meat becomes. Although the exact mechanisms leading to tenderness are not well understood, investigators associate both enzymatic protein degradation and the state of the actin/myosin interaction with meat tenderness (Goll, Geesink, Taylor, & Thompson, 1995; Koohmaraie, Kent, Shackelford, Veiseth, & Wheeler, 2002; Koohmaraie, 1992; Goll, Boehm, Geesink, & Thompson, 1998). Muscle is tender at first and then goes through a period during which it becomes tougher. At 24 to 36 h postmortem, the muscle starts to decrease in toughness and thus increase in tenderness (Wheeler & Koohmarie, 1994; Koohmaraie, Doumit, & Wheeler, 1996). The state of the actomyosin complex, which is formed through the binding of myosin and actin, is partly responsible for this toughness seen in rigor.

In the previous study in our lab, myofibrillar 1-D fingerprint analyses employing a combination of electrophoresis, three individual image analyses software packages, and



statistical analysis identified the concentration of seven peptides as being associated with, and predictive of, beef carcass tenderness (Sawdy, et al., 2004). In that study, mass spectrometric analysis identified the two most significantly contributing peptides as bovine myosin heavy chain fragments.

The objectives of this current research were: 1) to identify and characterize the next most significant protein/peptide in beef carcasses at 36 h postmortem associated with beef carcass tenderness after aging 7 days, 2) develop a more efficient system of image analyses employing a single software package, and 3) determine the relationship among the identified proteins/peptides.

## **Materials & Methods**

### *Steak samples*

Two 2.5-cm steaks were cut from twenty beef carcasses from randomly selected animals harvested at about 14 mo of age. These steaks were taken from the *longissimus dorsi* (*l. dorsi*) at the 12th rib at 36 h postmortem, and both steaks were vacuum-sealed in a 3-mil oxygen impermeable nylon/polyethylene vacuum pouch (Koch Supplies, Inc., Kansas City, MO). One steak from each animal was immediately frozen at -20 °C, whereas the other steak was aged for an additional 5.5 days at 4 °C and then frozen at -20 °C. The aged steaks were later used for Warner-Bratzler shear force (WBS) determination.

### *Warner-Bratzler shear force determination*

Sample steaks were thawed and allowed to equilibrate to an internal temperature of 4 °C. The steaks were cooked for 14 min using a Lincoln Inpinger conveyor oven (model# 1132-000-A),

which was set to 190.5 °C. An Ashcroft thermometer (model# ATFX 392 SKW) was used to measure the internal temperature of the cooked steaks. The cooked steaks were cooled to room temperature and six cores, 1.27 cm in diameter, were removed parallel to the muscle fiber orientation. Shear force values of the cores were acquired using a WBS apparatus (G-R Manufacturing, Manhattan, KS). The average WBS value of the six cores was calculated and used as the shear force values for each of the respective samples.

### *Myofibril preparation*

Myofibrils were prepared according to the method described by Goll, Young, and Stromer (1974) with the following modification. Starting material consisted of 0.2 g of tissue taken from the steaks that were frozen at 36 h postmortem. Sarcoplasmic proteins and fat were eliminated by repetitive centrifugation following extraction at 4 °C in standard salt solution (SSS): 0.1 M KCl, 0.02 M KH<sub>2</sub>PO<sub>4</sub>, 0.002 M MgCl<sub>2</sub>, 0.002 M ethylene glycol-bis[β-aminoethyl ether]- *N,N,N',N'*-tetraacetic acid (EGTA), 0.001 M NaN<sub>3</sub>, pH 6.8. Connective tissue was removed by filtration through nylon mesh.

### *Protein quantification*

Immediately after isolation, myofibrils were prepared for protein analyses by dialysis against SSS without EGTA. Samples were then diluted 1:1 with glycerol and stored at -20 °C. Protein concentrations of myofibrils were determined by a bicinchoninic acid (BCA) assay according to manufacturer's protocol (Pierce Endogen, Rockford, IL).

### *Sodium dodecyl sulfate polyacrylamide gel electrophoresis*

Extracted myofibrillar proteins were mixed with dissociation buffer (60 mM Tris buffer,

pH 6.8, containing 2% SDS, 15% glycerol, 350 mM DTT, and 0.1% bromophenol blue) and boiled, and 50 $\mu$ g of myofibrillar proteins were loaded on to each lane. Myofibrillar proteins were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli (1970), using a 1.5 mm  $\times$  12 cm  $\times$  14 cm polyacrylamide slab gel consisting of 10% resolving gel (30:0.8, acrylamide: *N*, *N*'-bis-methylene acrylamide) and a 3% stacking gel containing 1% SDS, at a constant voltage of 10 V cm<sup>-1</sup>. After electrophoretic separation, the gels were fixed overnight in 20% trichloroacetic acid. The gels were then washed in deionized water for 10 minutes. After washing, the gels were stained in Colloidal-Coomassie blue dye for 3 to 4 h and subsequently destained with several changes of water.

#### *Image Analysis*

Gel images were captured and digitized using a Microtek Scanmaker 5900 flatbed scanner (Microtek Inc. Carson, CA 90746). Molecular migrations of individual protein/peptide bands among samples were normalized and quantified using the built-in algorithms of Phoretix™ 1D software (Nonlinear USA Durham NC 27703). The molecular weights of protein/peptide bands were calculated by comparing the normalized migration patterns to known molecular weights of a broad range standard included in each gel. The percent that each band contributed to the total load of the sample, excluding the myosin and actin bands, was recorded.

#### *Statistical Analysis*

In order for a normalized protein/peptide band to be included in the analysis, it was required to contribute at least 1.5% of the total load of the sample, following the removal of the myosin and actin bands. The data were analyzed using a reverse stepwise linear regression according to Sawdy, et al. (2004). This analysis resulted in a model predictive of beef carcass tenderness. In

this model, seven protein/peptide bands between 20 and 160 kD were found to be significantly predictive of tenderness. To compare band percents of the three characterized proteins/peptides, a correlation was performed in SAS v. 8.0.

### *Mass spectrometry*

Mass spectrometry was performed at the Campus Chemical Instrument Center at The Ohio State University. One of the identified bands with a molecular weight of 18,671 Da was removed from the gel and submitted for primary sequence analysis by mass spectrometry as reported previously (Sawdy, et al., 2004).

## **Results and discussion**

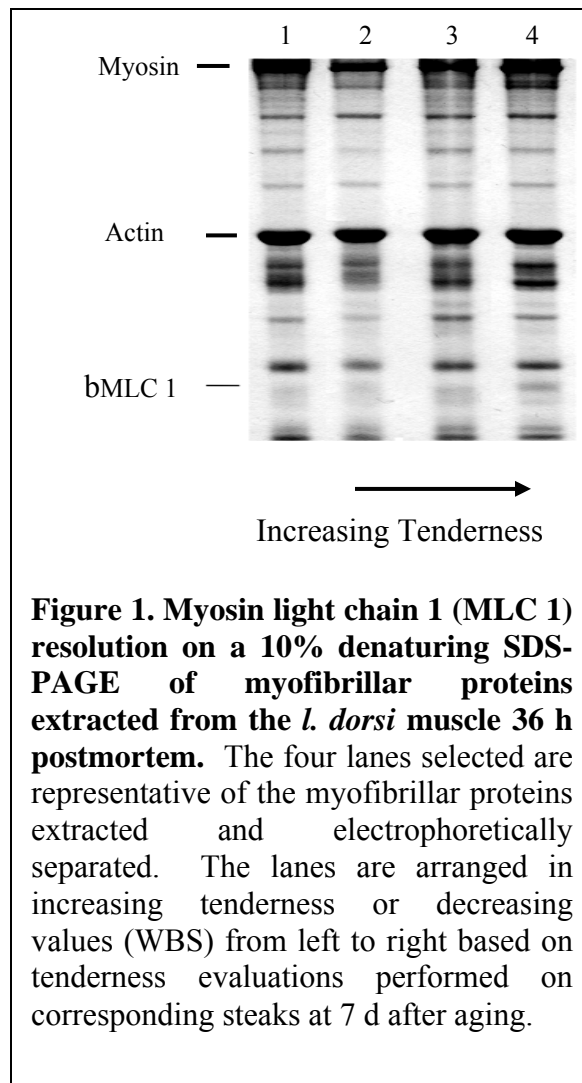
In Sawdy, et al. (2004), a predictive model for beef carcass tenderness was developed in which seven bands from muscle at 36 h postmortem were found to be associated with beef carcass tenderness at 7 d ( $r^2 = 0.82$ ). In that study, mass spectrometry characterized two of those bands as fragments of bMyHC. Five bands remained to be characterized. One of the goals of this study was to further characterize the protein/peptides associated with, and predictive of, beef carcass tenderness at 7d. The band investigated in this study was selected due to its high absolute value of  $t$  score from the regression analysis. A higher absolute value of  $t$  indicates a larger marginal importance for that element's involvement in the overall prediction model.

Fig. 1 is an electrophoretic image demonstrating the protein/peptide identified by the regression analysis that was submitted for sequence identification. A band corresponding to bMLC1, identified by mass spectrometry ( $P = 6.4 \times 10^{-15}$ ), increases in intensity, or concentration, with decreasing WBS from left to right. The primary sequence derived from the

mass spectrometric analysis and corresponding to bMLC1 is shown in Table 1. The sections that are underlined are the tryptic fragments characterized by mass spectrometry. In Fig. 1, the sample taken from a “tougher” steak on the left has a lesser quantity of bMLC1 than does the sample from the more tender steak on the right. This supports the hypothesis that cattle will yield carcasses with greater tenderness when the animals have higher amounts of bMLC1.

The proteins shown in Table 2 have been identified thus far in our lab as being positively associated with tenderness. The regression parameter estimates in Table 2 are negative because increased levels of the proteins/peptides are associated with lower WBS force value. Because these peptides are negatively associated with WBS, they are therefore positively associated with tenderness i.e., a low WBS value is indicative of a tender carcass. The  $|t|$  indicates the amount each particular band contributes to the overall model. As previously mentioned, a greater  $|t|$  indicates greater marginal importance to the final model. Therefore, the three largest positive contributors to tenderness according to the model, as indicated by the  $|t|$ , strongly implicate bovine myosin heavy chains and bovine myosin light chain 1 with beef carcass tenderness.

Fast skeletal bMyHCs are complexed with four bMLCs: two bMLC2s and, as yet, an unknown ratio of bMLC1 and bMLC3 (Fig. 2). The higher concentration or ratio of bovine myosin light chain 1, identified in the current study, joins the bMyHC fragments in implicating myosin’s participation in the mechanisms that determine tenderness. These results are consistent with, and expand upon, the results from previous studies that associated fiber type (based upon myosin isoform composition) with tenderness (Whipple, Koohmaraie, Dikeman, & Crouse, 1990).



**TABLE 1.** Sequence for bovine myosin essential light chain 1. Sections that are underlined and in bold are the tryptic fragments recognized by mass spectrometry

1	APAPAPPKEE	<u>KIDLSA</u> <b>IKIE</b>	FSK <u>QQQDEFK</u>	<u>EAFLLFDRTG</u>	ECK <u>ITLSQVG</u>
51	<u>DVLRALG</u> <b>TNP</b>	<u>TNAEVKKVLG</u>	NPSNEEMNAK	<u>KIEFEQFLPM</u>	<u>LQAISNNKDQ</u>
101	<u>GTIEDFVEGL</u>	<u>RVFDKEGNGT</u>	<u>VMGAELRHVL</u>	ATLGEK <u>MKEE</u>	<u>EVEALMAGQE</u>
151	<u>DSNGCIN</u> <b>YEA</b>	<u>FVKHIMSN</u>			

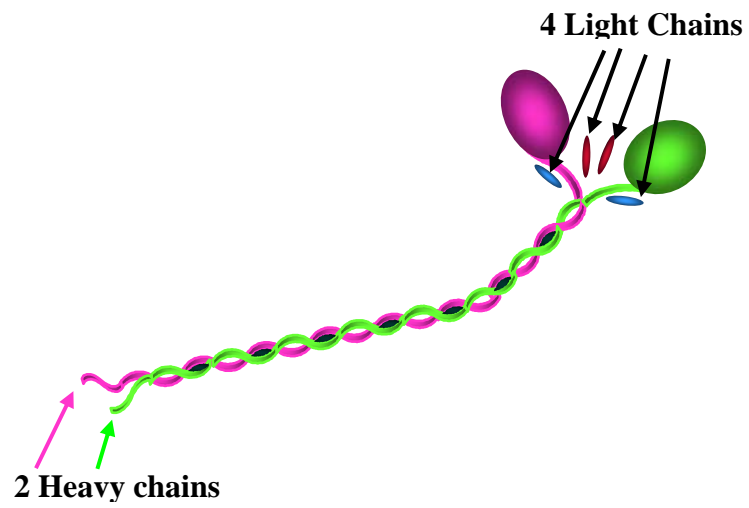
Accession # gi|1096612

**TABLE 2.** Proteins characterized that are associated with tenderness. A negative regression parameter estimate is associated with a lower WBS value. The absolute value of  $t$  indicates the marginal importance of each band.

<b>TABLE 2.</b> Protein/peptides associated with beef carcass tenderness after 7d aging.			
Band ID	Regression Parameter Estimates	SE of Estimates	$ t $
MHC Fragment	-4.14	1.12	3.70
MHC Fragment	-4.37	1.71	2.57
<b>MLC1</b>	<b>-3.81</b>	<b>1.66</b>	<b>2.30</b>



**Figure 2.** The myosin molecule is a hexamer composed of two heavy chains and four light chains.



To determine a possible connection between the identified proteins/peptides, a correlation procedure was performed. Bovine MLC1 was found to correlate positively ( $r = 0.50$ ) with both of the MyHC fragments ( $P < 0.05$ ). However, the two MyHC fragments were not correlated with each other. This suggests that the mechanism responsible for increased tenderness is dependent upon the MLC isoform associated with the MyHC. In the previous analysis on the two MyHC fragments, the sequence generated was insufficient to differentiate between MyHC isoforms. We hypothesize that each of the MyHC fragments is a different isoform and that both isoforms of MyHC respond similarly to MLC1 to increase tenderness.

The results on bovine tenderness from our lab are consistent with recent reports on swine tenderness. Both MyHC fragments and MLC1 have been associated with postmortem increases in pork quality and tenderness (Lametsch, Karlsson, Rosenvold, Andersen, Roepstorff, & Bendixen, 2003; Lametsch & Bendixen, 2001). Additionally, SDS-PAGE and 2D electrophoresis have indicated that MyHC is proteolyzed by  $\mu$ -calpain (Lametsch, Roepstorff, Møller, & Bendixen, 2004). Prior to this, researchers believed that MyHC did not undergo proteolysis by  $\mu$ -calpain in postmortem muscle (Koohmaraie, 1992).

Bovine MLC1 may play a critical role in the mechanisms of increased tenderness upon aging. Goll, Thompson, Taylor, and Christiansen (1992) and Goll, et al., (1995, 1998) have suggested that the actomyosin structure in postmortem muscle may be an important contributor to tenderness after aging through the weakening of the actomyosin interaction after 24 h postmortem. These studies have indicated that the N-terminus of MLC1 may influence the actin/myosin interaction (Goll, et al., 1995). According to Goll, et al. (1995), the N-terminus of MLC1 may contribute to the decline of the interaction between myosin and actin, resulting in a weakened actomyosin bond. This weakened bond may in turn contribute to increased tenderness by making myosin more susceptible to proteolytic degradation. This is speculated to occur 24-

36 hours postmortem and may be the outcome of proteolysis linked with MLC1.

Characterizing bMLC1 as another protein associated with tenderness helps expand our knowledge of the mechanisms of tenderness because it characterizes another peptide from the original seven found in our lab. The data in this manuscript, along with those previously reported by Sawdy, et al. (2004), strongly implicate the thick filament as being associated with beef carcass tenderness. This is consistent with and supports the hypothesis set forth by Goll et al. (1995, 1998) that bMLC1 potentially participates in events leading to increased tenderness with aging.

The results of this study could have direct effects on the beef industry, impacting several different groups within the industry. First, because the identity of the proteins would be known, this information could potentially lead to the development of breeding strategies based on tenderness data. This could eventually lead to cattle populations that produce carcasses with greater and more consistent tenderness. Next, marketers would know which products were more tender and hence more valuable. This would allow them to price the products accordingly. As a result, the carcasses could be separated into classes of economic importance, and a selective pricing system could be established. Consumers would also benefit because they would receive a more superior end-product. Finally, researchers profit because the identified peptides would increase our knowledge of the mechanisms underlying postmortem aging in muscle, what events are occurring, and how this is affecting tenderness.

## **Conclusion**

We have identified MLC1 as being associated with tenderness at 7 days postmortem. In addition we have found a correlation between MLC1 and MyHC fragments, which suggests a possible mechanism for increased tenderness due to fragmentation of MyHCs that have associated MLC1. Future research will examine the role that MLC1 plays in regulating MHC fragmentation postmortem.

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